

I CLAIM:

1. A method to reduce transcriptional interference between two or more tandemly arranged gene expression cassettes in a host cell comprising introducing into the host cell a polynucleotide comprising a) a first gene expression cassette encoding a first polypeptide, b) a spacer polynucleotide, and c) a second gene expression cassette encoding a second polypeptide, whereby the first gene expression cassette and the second gene expression cassette are positioned in a tandem orientation and the spacer polynucleotide of b) is positioned between the first expression cassette and the second expression cassette; and culturing the host cell under conditions, whereby transcriptional interference between the first gene expression cassette and the second gene expression cassette is reduced and the first polypeptide and the second polypeptide are expressed.

2. The method according to claim 1, wherein the positioning of the first gene expression cassette and the second gene expression cassette is in an orientation selected from the group consisting of head (5')-to-tail (3') orientation, head (5')-to-head (5') orientation and tail (3')-to-tail (3') orientation.

3. The method according to claim 1, wherein the spacer polynucleotide of b) is selected from the group consisting of:

20 (i) a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 1;

(ii) a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 2;

(iii) a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 3; and

(iv) a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 4.

4. The method according to claim 1, wherein the spacer polynucleotide of b) comprises at least a 40% adenine and thiamine nucleotide content.

30 5. The method according to claim 1, wherein the spacer polynucleotide of b) comprises at least a 46% adenine and thiamine nucleotide content.

6. The method according to claim 1, wherein the spacer polynucleotide of b) comprises at least a 48% adenine and thiamine nucleotide content.

7. The method according to claim 1, wherein the spacer polynucleotide of b) comprises at least a 63% adenine and thiamine nucleotide content.

5 8. The method according to claim 1, wherein the host cell is selected from the group consisting of a bacterial, fungal, yeast, plant, animal and mammalian cell.

9. The method according to claim 8, wherein the plant cell is selected from the group consisting of an apple, *Arabidopsis*, bajra, banana, barley, bean,
10 beet, blackgram, chickpea, chili, cucumber, eggplant, favabean, maize, melon, millet, mungbean, oat, okra, *Panicum*, papaya, peanut, pea, pepper, pigeonpea, pineapple, *Phaseolus*, potato, pumpkin, rice, sorghum, soybean, squash, sugarcane, sugarbeet, sunflower, sweet potato, tea, tomato, tobacco, watermelon, and wheat cell.

15 10. The method according to claim 1, wherein at least one of the gene expression cassettes comprises a polynucleotide encoding a polypeptide selected from the group consisting of an antigen, an alpha-amylase, a phytase, a glucanase, a xylase, an insect resistance, a nematode resistance, a fungus resistance, a bacterium resistance, a virus resistance, an abiotic stress resistance, a
20 nutraceutical, a pharmaceutical, an amino acid content modifying, a herbicide resistance, a cold tolerance, a drought tolerance, a heat tolerance, and an antioxidant polypeptide.

11. A host cell produced by a method comprising:
25 (a) introducing into the host cell a polynucleotide comprising (i) a first gene expression cassette encoding a first polypeptide, (ii) a spacer polynucleotide, and (iii) a second gene expression cassette encoding a second polypeptide, whereby the first gene expression cassette and the second gene expression cassette are positioned in a tandem orientation and the spacer polynucleotide of (ii) is positioned between the first expression cassette and the second expression cassette; and
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- (b) culturing the host cell under conditions, whereby transcriptional interference between the first gene expression cassette and the second gene expression cassette is reduced and the first polypeptide and the second polypeptide are expressed.
- 5 12. A non-human organism comprising the host cell of claim 11.
13. The non-human organism according to claim 12, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, a plant, an animal, and a mammal.
14. The non-human organism according to claim 12, wherein the non-
10 human organism is selected from the group consisting of an apple, *Arabidopsis*, bajra, banana, barley, bean, beet, blackgram, chickpea, chili, cucumber, eggplant, favabean, maize, melon, millet, mungbean, oat, okra, *Panicum*, papaya, peanut, pea, pepper, pigeonpea, pineapple, *Phaseolus*, potato, pumpkin, rice, sorghum, soybean, squash, sugarcane, sugarbeet, sunflower, sweet potato,
15 tea, tomato, tobacco, watermelon, and wheat plant.